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TNO report

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Sleep and Alertness management II: Effects on sleep pattern and sleep quality in marmosets

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Slaap- en alertheidsmanagement II: Effecten op slaapritme en slaapkwaliteit in Marmosetapen

Probleemstelling

In opdracht van het Ministerie van Defensie wordt door TNO Defensie en Veiligheid onderzoek gedaan naar praktische richtlijnen om tijdens militaire missies ernstige vermoeidheid te voorkomen en de prestaties en alertheid te optimaliseren. Ervaringen met militaire missies hebben namelijk geleerd dat de nadelige effecten van slaaptekort een zeer belangrijke rol speelden bij de uitvoering van de missies. Aangezien tijdens militaire operaties de omstandigheden door omgeving en door de aard van de missie niet altijd optimaal zijn om spontaan de slaap te vatten zou het gebruik van kortwerkende slaapmiddelen een oplossing kunnen bieden.
Deze slaapmiddelen moeten niet alleen de inslaaptijd verkorten en bespoedigen, maar moeten ook garant staan voor een goede slaapkwaliteit. Deze kwaliteit kan gemeten worden aan de hand van de slaaparchitectuur.

Beschrijving van de werkzaamheden

In een eerder uitgevoerd onderzoek voor het Ministerie van Defensie is een selectie gemaakt van mogelijk geschikte slaap- en alertheidsverhogende middelen (Busker *et al.*, TNO rapport PML 2000-A2). In het hier gerapporteerde onderzoek zijn van deze geselecteerde kortwerkende slaapmiddelen temazepam, zolpidem en zaleplon de effecten op de slaapkwaliteit in een relevant diermodel onderzocht. Slaapkwaliteit heeft betrekking op het intact houden van de normale fasen zoals die in natuurlijke slaap worden gezien. Hiervoor is het slaapmodel in de marmosetaap ontwikkeld waarna vervolgens de slaapkwaliteit van de slaapmiddelen volgens gevalideerde methoden, welke ook in de kliniek worden toegepast, is getest.
Deze methoden maken gebruik van de hersenactiviteit, het elektroencephalogram (EEG), waarbij de verschillende slaapfases zijn te onderscheiden.

Resultaten en conclusies

De resultaten van het EEG en de slaapfases op hersenniveau laten zien dat de marmosetaap een goed model is voor slaaponderzoek. Daarnaast zijn er geen grote verschillen gevonden tussen de effecten van de verschillende slaapmiddelen temazepam, zolpidem en zaleplon op de kwaliteit van de slaap. Alle drie de slaapmiddelen lijken de kwaliteit van de slaap op een positieve wijze te beïnvloeden. Tijdens de eerste helft van de nacht was de slaap dieper dan normaal. In een aantal gevallen werden echter ook 'rebound' -effecten waargenomen. Alle slaapmiddelen, maar vooral temazepam, resulteerden in zogenaamde 'carry-over'-effecten, dat wil zeggen dieren dreigden 's ochtends na ontwaken weer in slaap te vallen. Dit is een algemeen verschijnsel bij slaapmiddelen. Na gebruik van temazepam werden ook veel 'slaap spindles' in het EEG gezien, welke de kans op ontwaken door sensorische input, zoals geluiden van buitenaf, verkleinen. Dit wordt daarom beschouwd als een positief effect. Dit was ook terug te vinden in het minder ontwaken tijdens de nacht na gebruik van temazepam los van facetten als snelheid en duur van de werking. Dit alles suggereert dat voor het management van slaap en alertheid in een militaire setting alle drie de slaapmiddelen temazepam, zolpidem en zaleplon van waarde kunnen zijn. De voorkeur met betrekking tot verbetering van slaap-kwaliteit op EEG-niveau heeft temazepam. Echter, dit betekent niet dat dit middel ook het meest effectief is om, na ontwaken, ook de inzetbaarheid te kunnen optimaliseren. In twee volgende rapporten zal het effect van slaap- en alertheids-management op functioneren besproken worden.

Toepasbaarheid

Informatie over de verstoring of verbetering van het normale slaappatroon is zinvol om tot een advies te komen over de inzetbaarheid van farmaca voor het optimaliseren van slaap- en alertheids-management. Aangezien deze middelen aan gezonde mensen zonder pathologische slaapproblemen zal worden voor-geschreven, mag het gebruik van deze middelen de normale slaaparchitectuur niet verstoren. Dit onderzoek op basis van het slaappatroon als functie van de hersenen is bovendien zinvol voor de extrapolatie van

de resultaten naar de effectiviteit van de middelen naar de mens.

Projectafspraken

Dit onderzoek maakt deel uit van een project waarin de bruikbaarheid van farmacologische middelen voor het optimaliseren van slaap en alertheid wordt onderzocht. In een eerder rapport is de farmacokinetiek van de geselecteerde slaap- en alertheidsverhogende middelen beschreven (TNO-DV 2006 A268; TD2006-056). In de volgende rapporten zal de invloed van de slaapmiddelen op het functioneren (TNO-DV 2006 A270; TD2006-058) en de invloed van alertheidsverhogende middelen op het functioneren op momenten dat het circadiane ritme slaap dicteert (TNO-DV 2006 A271; TD2006-059) onderwerp van aandacht zijn.

PROGRAMMA	PROJECT
Programmabegeleider dr. J. van der Plas, MGFB/MGFB, Staf/beleidsondersteuning	Projectbegeleider Kol. Vliegerarts J.L.A. van der Hoorn, Commando Luchtstrijdkrachten/Hoofd Afdeling Gezondheidszorg operaties
Programmaleider P.J.L. Valk, TNO Defensie en Veiligheid, Business Unit Human Factors, Afdeling Human Performance	Projectleider dr. I.H.C.H.M. Philippens, TNO Defensie en Veiligheid, Business Unit Biologische en Chemische Bescherming, Afdeling Diagnose en Therapie
Programmatitel Arbeidsomstandigheden bij Defensie	Projecttitel Slaap- en alertheidsmanagement
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Frequentie van overleg Met de programma/projectbegeleider werd acht maal gesproken over de invulling en de voortgang van het onderzoek.	Projectteam R.A.P. Vanwersch, M. Jongsma, B. Groen, C. Kersten, A. Strijkstra, dr. R.W. Busker, dr I.H.C.H.M. Philippens.

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Samenvatting

In deze studie is de marmosetaap als model voor het meten van effecten op de slaapkwaliteit door middel van electroencefalogram (EEG) metingen gevalideerd. Vervolgens zijn de kort werkende slaapmiddelen temazepam, zolpidem en zaleplon getest in hoeverre deze de kwaliteit van de slaap en/of de normale slaap cyclus/architectuur verstoren in de marmosetaap. De resultaten laten zien dat de marmosetaap een goed model is voor slaaponderzoek. Verder zijn er geen grote verschillen gevonden tussen de effecten van de verschillende slaapmiddelen op de kwaliteit van de slaap. Alle drie de middelen lijken de kwaliteit van de slaap op een positieve wijze te beïnvloeden. Maar er werd in een aantal gevallen ook 'rebound effecten' waargenomen. Alle slaapmiddelen, maar vooral temazepam, resulteerden in zogenaamde carry-over effecten, dat wil zeggen dieren dreigden 's ochtens na ontwaken weer in slaap te vallen. Na temazepam werden ook veel slaap 'spindles' in het EEG gezien, welke de kans op ontwaken door sensorische input, zoals geluiden van buiten af, verkleinen. Dit bleek ook uit het minder frequent ontwaken tijdens de slaap na gebruik van temazepam. Daarnaast, zijn er aanwijzingen dat zolpidem ongewenste effecten heeft bij gebruik door vrouwen. Dit alles suggereert dat voor het management van slaap en alertheid in een militaire setting de slaapmiddelen temazepam en zaleplon beiden van waarde kunnen zijn, waarbij de voorkeur bij temazepam ligt.

Summary

In this study, the marmoset monkey model was validated using nocturnal electroencephalogram measurements for evaluating effects on sleep quality. In order to test whether the proposed sleep inducing drugs affect the quality of sleep and/or disrupt the normal sleep cycle/architecture, the effects of the short acting hypnotic drugs temazepam, zolpidem and zaleplon on sleep were determined in the marmoset monkey. The results showed that the marmoset monkey model is a valid model for the research of sleep. Furthermore, no large differences between the effects of the tested sleep inducing drugs on the quality of sleep could be observed. All three drugs tended to affect the quality of sleep in a positive way, but also induced some 'rebound-effects'. All drugs, but temazepam especially, resulted in some carry-over effects, i.e. after awakening animals tended to fall asleep again. On the other hand, after temazepam sleep spindles were observed often. These spindles lower the chance of awakening as a result of sensory input like noise. This was in agreement with the finding that after temazepam less frequent wakening was observed. Furthermore, there some indications that zolpidem has undesired effects in women. This might indicate that for the management of sleep in a military setting the sleep inducing drugs temazepam and zaleplon might both be useful, with the preference for temazepam.

Contents

1 Introduction

Regular and good quality sleep is vital for proper performance and a healthy life. Disturbed sleep is hazardous and can have multiple causes. In a military setting situations that induce disturbed sleep frequently occur: round the clock activity requires rapid work shift changes and long night duties, and provokes sleep loss and high stress levels. This may result in excessive sleepiness. These situations have been documented in a separate TNO report [Simons and Valk, 1999]. Recommended possible solutions with direct usefulness in crew endurance plans included strategic napping, chronobiological treatments and the use of sleep-inducing and wake-promoting drugs.

Any pharmacological intervention may result in unwanted side-effects. Hypnotic drugs are a class of drugs that induce sleep, but may also cause undesired carry-over effects, such as excessive sleepiness after sleep when wakefulness is required. For sleep and alertness maintenance this means that a combination of a short acting hypnotic drug and a fast acting stimulant drug may be necessary in some situations.

An overview of sleep-inducing and wake-promoting drugs to aid sleep and to enhance alertness during military service has been given in other TNO reports [Simons and Valk, 1999; Busker *et al.*, 2000]. The Busker *et al.* [2000] report includes a literature study on the use of animal models for human sleep-wake management, and a theoretical evaluation of potential candidate, e.g. hypnotics ('downers') for sleep induction and stimulants ('uppers') for wake maintenance.

In situations when sleep needs to be induced by means of drugs, it is important to know that the induced sleep is not of a lower quality than normal sleep. The quality of sleep can be investigated by means of the investigation of the sleep architecture.

To investigate effects of drugs, animal studies are in several respects preferable over human studies. Drug research on human volunteers has practical and ethical problems, and more invasive studies are not possible at all in humans. Therefore, the effects of sleep induction have been tested in an animal model mimicking human sleep patterns as close as possible: the marmoset monkey.

Although in many ways comparable, a number of differences have been reported between sleeping patterns in man and in primates (see Table 1). One of the differences in the sleep pattern of the marmoset monkey is the duration of the sleep cycles: 45 9 minutes instead of 90 minutes. However, this aspect has no effect on the architecture of sleep, which resembles the human sleep rhythm (see Table 1).

Table 1 Phylogenetic aspects of sleep stages.

Species	Total sleep time h	REM %	Delta-sleep %
Human	6-11	20	20
Chimpanzee	12-14	15-23	20
Rhesus	6-12	15-20	36
Marmoset	8-12	13	20

Data partly adapted from Lagarde [1990] and partly collected from our own data.

In this study, the marmoset monkey model was validated using nocturnal electroencephalogram measurements to measure effects on the sleep quality. In order to test whether the proposed sleep inducing drugs affect the quality of sleep and/or disrupt the normal sleep cycle/architecture, the effects of the short acting hypnotic drugs temazepam, zolpidem and zaleplon on sleep were determined in the marmoset monkey.

2 Theory

2.1 Sleep as an active and rhythmical process

Till the 50s the research on sleep was dominated by a passive theory of sleep. This theory described sleep as a process which sets in when there is not enough stimulation; or as a shutdown of several systems which are active during wakefulness. Nowadays researchers are convinced this is not the case. Sleep is believed to play an active role and sleep deprivation induces several disruptions of important functions [Siegel, 2005]. Currently there are three theories regarding the function of sleep:

1 sleep as a recovery period for the brain;
2 sleep as a organization mechanism for the information gained during wakefulness;
3 sleep as a process for the creation of new strategies and combinations of stored information.

These three theories can be summarized by one single function of sleep: sleep is needed to sustain (the abilities of) the central nervous system.
And as a consequence it is needed for a healthy life and normal performance.

Several electrical potentials can be measured in order to monitor sleep.

- The ElectroEncephalogram (EEG), measuring differences in potentials from the brain cortex.
- The ElectroRetinogram (ERG): measuring differences in potentials from the eye muscles. These eye muscles are involved during rapid eye movement (REM) sleep.
- The ElectroMyogram (EMG): measuring differences in potentials from muscles, for instance the chin muscle. Besides the eye muscles also other muscles are involved during REM sleep.

2.2 Sleep architecture

Research showed that for humans there is a particular structure in the distribution of sleep phases during the night [Carskadon and Dement, 1994]. Visually, the different sleep phases can be separated by careful observation and judgment of the EEG, EMG and the level of activity. There is some variation in the definition of the different sleep phases, but in general six different sleep phases are described.

Phase 0	Wakefulness: the EEG mostly consists of so-called alpha and beta waves (see Figure 1).
Phase I	Drowsiness: usually short lasting, the EEG mostly consists of alpha, beta and theta waves.
Phase II	Light sleep ('Unequivocal sleep'): the EEG mostly consists of theta rhythm and sometimes also alpha rhythm (see Figure 1). Notably, the first sleep spindles and K-complexes occur. These spindles are presumably generated by the thalamic nuclei. The K-complexes are indications for the transition to the deep sleep. The EMG signal shows some activity.
Phase III	Deep sleep: The EEG mostly consists of high amplitude, low frequency waves, the so-called delta waves. These delta waves are present in 20-50% of the EEG (see Figure 1).

Phase IV	Cerebral sleep: The EEG consists of a minimal level of 50 % delta waves.
REM sleep	Rapid eye movement (REM) sleep, also called paradoxical sleep since during this phase an 'active' EEG signal can be observed, i.e. low amplitude high frequency waves (see Figure 1), and the EMG signal shows (almost) no potential.

Phases III and IV together are also referred to as slow wave sleep (SWS) or delta sleep. The delta sleep is needed for physical recovery and its presence appears to be consistent independent of a short- or a long sleep period. When this delta sleep phase is affected, people will experience poor sleep quality. REM sleep is associated with high levels of neuronal activity and dreaming. Although the real purpose of REM sleep is unknown, it is thought to be important for memory and learning processes and/or to pre-stimulate the brain before awakening to facilitate effective sensory and motor function [Siegel, 2005].

Figure 1 shows representative EEG signals for the different phases of sleep.

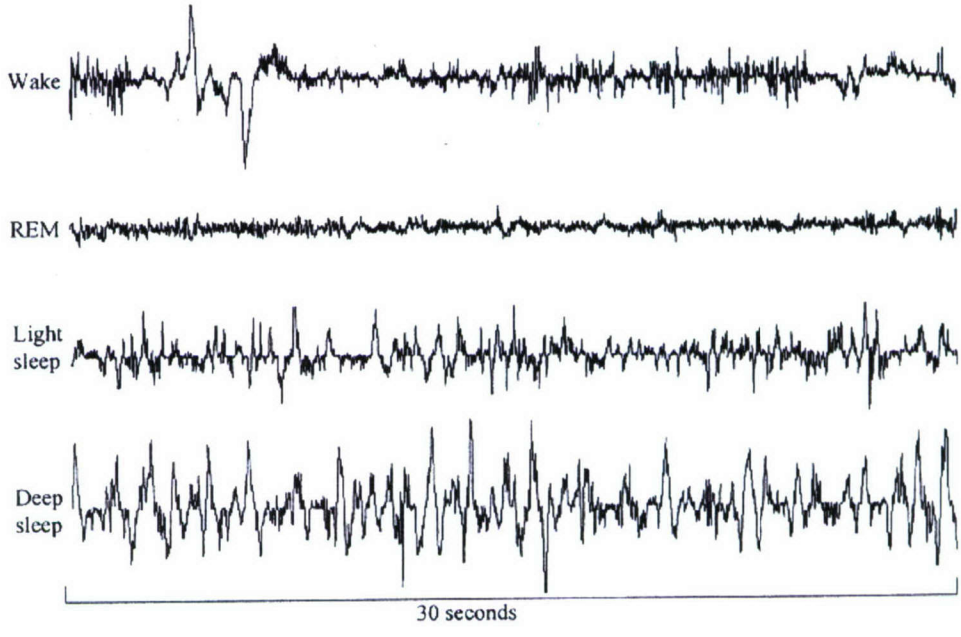

Figure 1 EEG signals during the different phases of sleep.

An average human sleep cycle has a duration of about 90 minutes. At the beginning of the night a larger percentage of these cycli are spent in delta sleep, while later in the night more light sleep and REM sleep occur [Carskadon and Dement, 1994].

The sleep architecture can be represented in a hypnogram, where the x-axis shows the time and the y-axis shows the presence of the different sleep phases. Figure 2 shows an example of a hypnogram.

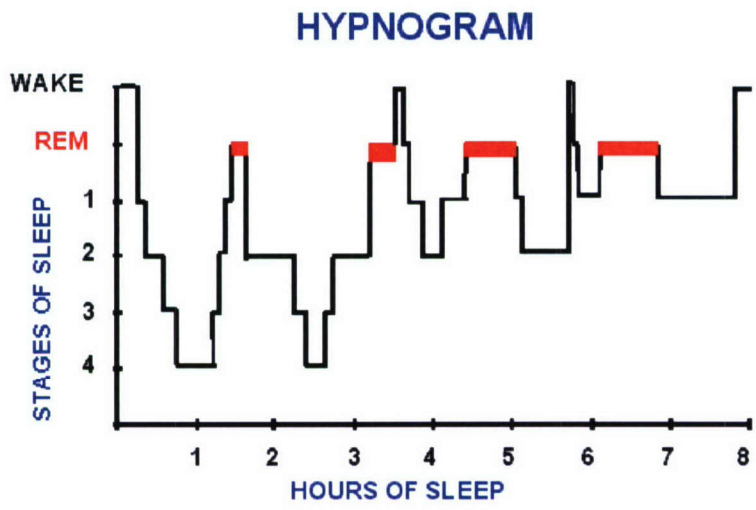

Figure 2 A simplificated example of a human hypnogram. 1: light sleep (phase I), 2: light sleep (phase II), 3: delta sleep (phase III), 4: delta sleep or deep sleep (phase IV), REM: rapid eye movement sleep, WAKE: wakefulness.

2.3 EEG during sleep

A minimum number of two electrodes are needed in order to record an EEG signal: an active electrode, which is placed near a location where the brain activity of interest is generated and an indifferent-electrode which is placed at some distance from the active electrode. The difference in measured potentials between the two electrodes is measured. In general, in humans potential differences ranging from 20 to approximately 100 μV are measured, as is the case in marmoset monkeys. However, in humans the signals are measured at the level of the scalp whereas in marmoset monkeys the signal is measured intracranially through the skull on the *dura mater*.

The EEG signal can be divided in a series of waves of different frequencies [Muthuswamy and Thakor, 1998]. These series of waves of different frequencies observed in the EEG can be divided into so-called frequency bands. In general, the following frequency bands are described.

Delta waves:	These waves have a frequency in the range of 0.5-4 Hz and are generally associated with sleep.
Theta waves:	These waves have a frequency of 5-7 Hz and are also associated with sleep.
Alpha waves:	These waves have a frequency in the range of 8-13 Hz and are generally associated with a state of relaxed wakefulness.
Beta waves:	These waves have a frequency in the range of 13-30 Hz and are generally associated with wakefulness.

3 Materials and methods

3.1 The animal model

Sleep in other mammals is in many respects similar to that of humans. Rodent systems, e.g. mice and rats, have however some limitations. Their sleep patterns are poly-phasic and they sleep primarily during the day. This deviates considerably from the consolidated mono-phasic night-time sleep in humans. Sleep patterns with the best resemblance to human sleep are found in primates.

In our laboratory, sleep patterns of marmoset monkeys (*Callithrix jacchus*) have been studied. These animals sleep mono-phasically during the night and have very similar sleep EEG variables, including a sleep intensity decline during the night, regular REM sleep episodes with relatively long cycle duration. Furthermore, experience exists with different read-out systems on behavioral performance [Philippens *et al.*, 2000], which have been used to test the sleep-alertness conditions of the animals.

The marmoset monkey (Figure 4) is a small size monkey which facilitates the handling of the animal. In general, they are about 20 cm tall and weigh approximately 300-500 g.

The marmoset monkey has a spontaneous day-night cycle of 23.2 ± 0.3 hours, and similar to man light and social events are important factor that might influence sleep [Duffy *et al.*, 1996]. Also, the EEG patterns of marmoset monkeys during the sleep phases match those observed in humans. Figure 3 clearly shows that during sleep (NREM) the EEG is dominated by low frequency waves (delta waves), while during REM sleep and wakefulness the EEG consists mostly of high frequency waves, as is the case in humans.

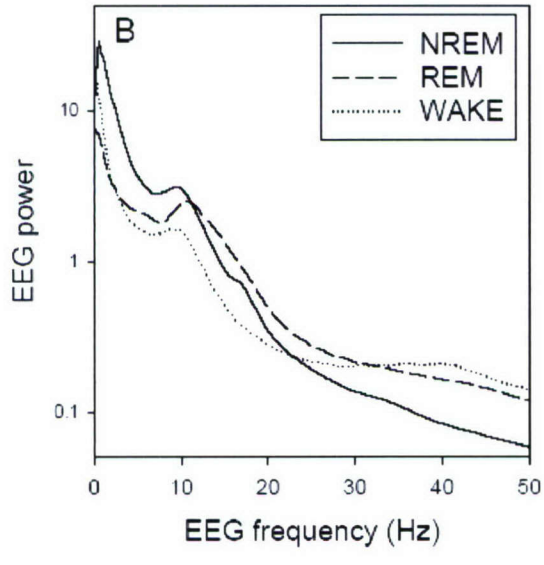

Figure 3 The spectrograms of EEG recordings of marmoset monkeys for different phases of the sleep-wake cycle. NREM: non-REM sleep, REM: REM sleep, WAKE: wakefulness. [Philippens *et al.*, 2004].

In the present study six marmoset monkeys (*Callithrix jacchus;* see Figure 4), aged 2-6 years with initial body weights between 350-500 g obtained from Harlan, United Kingdom, were used to validate the animal model and to investigate the effects of the three sleep inducing drugs using EEG and EMG measurements during sleep, and also the recovery of these effects was investigated the night after the night of administration.

Figure 4 A picture of a marmoset monkey (*Callithrix jacchus*).

The monkeys were housed separately in cages (61 x 61 x 41 cm). The ambient temperature in the housing room was regulated at 25 ± 2 °C and the relative humidity was maintained at > 60%. In this room a 12-hour day and night cycle was maintained. However, on the nights of sleep deprivation the light was kept on during the night. Daily they were fed with pellet chow, peanuts, fruit, boiled egg, baby biscuits, sunflower seeds, bread, beans, and fruit syrup after training or testing. Water was available *ad libitum*.
All aspects of animal care are described in Standard Operating Procedures, which are in agreement with current guidelines of the European Community. The independent TNO committee on Animal Care and Use approved all protocols for the animal experiments.

3.2 Drugs

Sleep inducing drugs which have a short lasting effect and hardly any residual effects are preferred for the regulation of sleep since these drugs do not disturb the performance the following day. Also, the preferred drug induces its effect quickly after administration.

For the present study three sleep inducing drugs have been selected: temazepam (TMP), zolpidem (ZPD) and zaleplon (ZAL). The effects of these drugs on the sleep architecture were investigated. Also, the recovery of the drug effects the night after the night of administration was measured.

3.2.1 Temazepam

The most frequently prescribed hypnotic drugs by far are the benzodiazepines. Temazepam (TMP) is a C_3-hydroxylated derivative of the benzodiazepine diazepam. The plasma elimination half-life in humans is 10-12 hours [Jochemsen *et al.*, 1983; Lockniskar and Greenblatt, 1990]. Peak plasma levels are achieved after approximately 70 minutes [Jochemsen *et al.*, 1983]. These relatively favourable pharmacokinetics contribute to the fact that this drug is prescribed quite often. However, TMP does induce mild reductions in the performance the following morning.

TMP increases the occurence of delta sleep and also increases the amount of sleep spindles. TMP does not seem to affect the REM sleep. A study in which sleep was induced during daytime 20 mg TMP was shown to be effective, but did not result in disruptions of the sleep architecture [Porcu *et al.*, 1997a; Porcu *et al.*, 1997b].

3.2.2 *Zolpidem*

Zolpidem (ZPD) is an imidazopyridine which differs in structure from the benzodiazepines and is a rapid and short acting hypnotic. It is rapidly absorbed after oral administration and is approximately 92% bound to plasma proteins. In humans, the major metabolic routes include oxidation and hydroxylation; none of the metabolites appears to be pharmacologically active. After a single 10 mg oral dose, a peak plasma concentration of 125 µg/L occurs 1.7 hours post dose, which extrapolates to a terminal elimination half-life time of 2 hours [Greenblatt *et al.*, 1998].

ZPD is commonly used for short-term treatments of insomnia, and has been FDA approved since 1993. Since ZPD selectively binds to the omega-δ subtype of the GABA receptors, it does not induce muscle relaxation and/or sedation [Hoehns and Perry, 1993] in contrast to most benzodiazepines.

3.2.3 *Zaleplon*

The relatively new hypnotic zaleplon (ZAL) is a non-benzodiazepine derivative and was developed to be safer than the earlier developed conventional hypnotics, causing fewer side effects. Similar to ZPD, ZAL has a selective affinity for the omega-1 subtype benzodiazepine receptor.

After oral administration of 10 mg ZAL in humans, a maximum plasma level was reached after 0.76 hour with a maximum concentration of 37 µg/l; the elimination half-life time is approximately 46 minutes [Rosen *et al.*, 1999; Sanchez Garcia *et al.*, 2000].

3.2.4 *Effective doses*

The effective dose for the induction of sleep in marmoset monkeys (see Table 2) were determined per drug by using a literature search, behavioral observations and pharmacokinetic analyses of the compounds. These pharmacokinetics are described in a separate report (TNO-DV 2006 A268).

Table 2 Effective doses in marmoset monkeys and the resulting pharmacokinetic values.

Drug	Dose (mg/kg)	$t_{1/2}$ (min)	C_{max} (µg/ml)	t_{Cmax} (min)
TMP	15	46	0.22	74
ZPD	3	117	0.35	62
ZAL	10	33	0.32	49

3.3 EEG and EMG measurements

3.3.1 *Data acquisition*

The EEG and EMG signals were measured telemetrically using a two-channel transmitter (Model TA10CA-F40, Data Sciences International, USA; see Figure 5). Each channel displayed the signal (in µV) measured between two intracranially implanted electrodes. The existing transmitters, originally designed as implants, were

relatively large and had to be adjusted in size to allow for external use with marmoset monkeys. The end result was a small non-permanent external transmitter (see Figure 5).

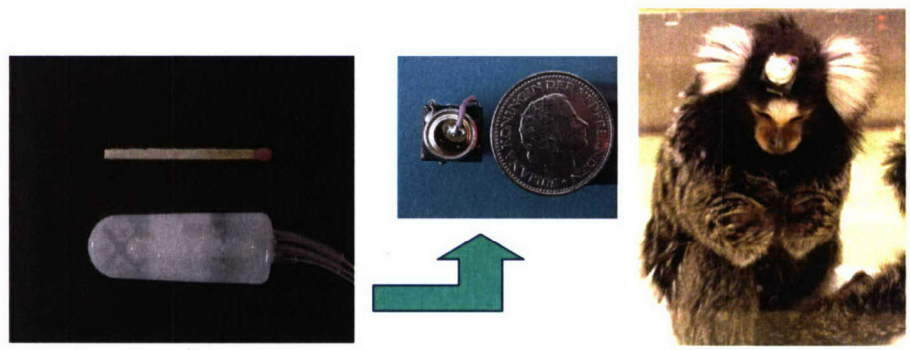

Figure 5 The original (implant) transmitter (left) and the adjusted transmitter which allows for external use with marmoset monkeys (middle). The original transmitter was an implantable one, whereas the adjusted transmitter is a non-permanent external transmitter which can be used to record the EEG and EMG of a freely moving marmoset (right).

The EMG electrodes were implanted in the neck and chin. Especially the chin muscle is of importance in sleep studies since this is the last muscle to seize activity when REM sleep starts. The relatively frontal placement of the EEG electrodes (see Figure 6), allows for a good measurement of the activity of the frontal cortex. This frontal cortex shows alpha rhythm activity mostly during sleep instead of during wakefulness. Also, the most frontally placed electrode could possibly also measure the activity of the eye muscle.

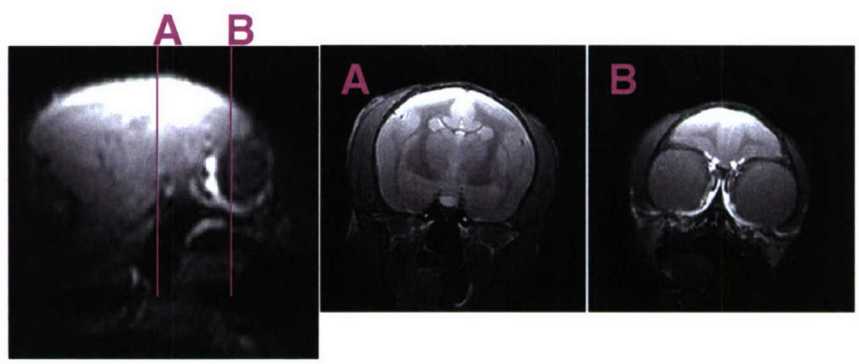

Figure 6 Electrode placement: The picture on the left shows a sagital cross-section using magnetic resonance imaging (MRI). Line B lies anterior of line A. The two pictures on the right show cross-sections at the location of lines A and B. The electrodes were placed on the skull at these locations and 2 mm lateral of the midline.

The EEG and EMG signals are recorded using a system by Data Sciences International (DSI, a division of Transoma medical, Arden Hills, USA). The signals are recorded during the first 55 seconds of every minute and stored on the computer during the last 5 seconds of each minute. After the recordings the data was transferred for analyses using Polyman (MCH, The Hague, The Netherlands) and Microsoft Excel (Microsoft corporation, USA).

3.3.2 *Data analyses*

The raw EEG and EMG signals were used for manual scoring of the sleep phases using the analysis program Polyman, according to Rechtschaffen and Kales [1968].
The analysis program Polyman was made at the Centre for sleep and wake disorders in The Hague (MCH), where it is used in the clinic. By using the same program the extrapolation towards the human situation is facilitated. Also, the MCH had an advisory role regarding the overall setup and analysis techniques used in this study. Observations of video recordings of the animals during the night were used to verify the scored sleep stage in case of uncertainty.
The obtained scoring results were used to construct hypnograms. First, for the data of the entire night the hypnograms were constructed and the percentages of time per sleep phase were calculated. Since the sleep inducing drugs all have short lasting effects, the data of the night were divided into two halves. It was expected that the effects of the drugs were most pronounced in the data of the first half of the night.

The obtained results were statistically tested using independent samples T-tests (SPSS inc, Chicago, USA). A p-value of <0.05 was considered to be statistically significant.

4 Results

4.1 Sleep architecture

4.1.1 Hypnograms

Figure 7 shows an example of the obtained hypnograms.

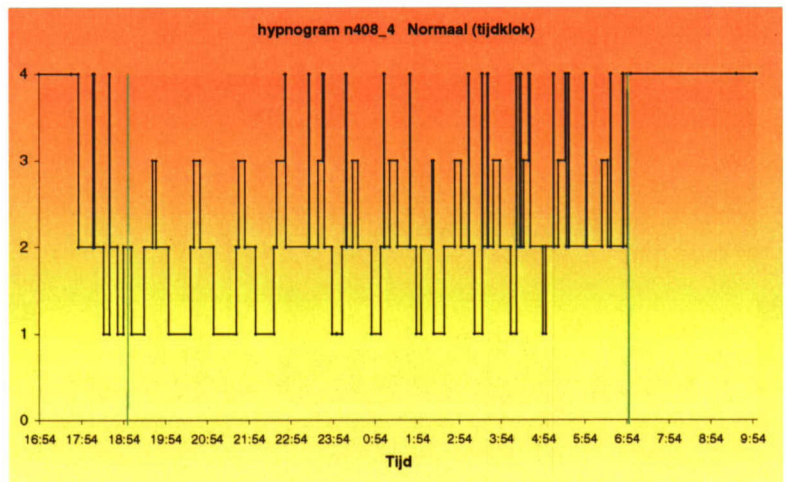

Figure 7 A hypnogram obtained in a marmoset monkey under normal circumstances. On the x-axis the time is displayed and on the y-axis the sleep phases are shown. (1: Deep sleep; 2: Light sleep; 3: REM sleep; 4: Wakefulness) The green lines indicate the times were the light switches off and on.

The hypnograms showed a clear cyclic pattern. A cycle often started with light sleep, after which the animal falls into deep sleep, usually followed by a REM-phase. After this REM phase the animal shortly regained wakefulness, after which a new cycle started. Such a cycle lasted approximately 45 minutes in the marmoset monkeys.

4.1.2 Sleep phases per night in percentages

Table 3 shows the average percentages per sleep phase observed in the marmoset monkeys during a control night as well as after the drugs.

Table 3 The average percentages per sleep phase (n=6). The human percentages [Carskadon and Dement, 1994] are displayed for comparison purposes.

Hypnogram	Delta sleep	Light sleep	REM-sleep	Wakefulness
Baseline	20.3	58.3	12.3	9.0
TMP	20.9	62.9	11.2	5.1
ZPD	21.1	57.9	13.9	7.1
ZAL	20.1	59.6	12.9	7.4
Human	13-23 %	47-60 %	20-25 %	5 %

When considering the data from the entire night no statistical significant differences could be observed. However, due to the short-lasting effects of the drugs, effects were mostly expected in the beginning of the night. Therefore, the data were divided into two parts (before 0.00 h and after 0.00 h). The percentages per sleep phase during the first and second half of the night are displayed in Table 4. During the baseline measurements

a higher percentage of delta sleep was observed during the first part of the night, which is in accordance with findings in humans [Carskadon and Dement, 1994] and results from another study in marmoset monkeys [Crofts *et al.*, 2001]. Also, the percentage of wakefulness during the first half of the night clearly decreased after the sleep inducing drugs, as was expected.

Tabel 4 Percentages per sleep phase during the first and second half of the night.

Treatment	Delta sleep		Light sleep		REM-sleep		Wakefulness	
Half	first	second	first	second	first	second	first	second
Baseline	29.0	11.2	49.8	66.3	8.9	14.9	12.3	6.4
TMP	37.1	10.3	51.2	69.3	7.7	13.5	4.0	7.0
ZPD	36.4	8.0	44.4	69.9	13.0	14.4	5.8	7.8
ZAL	36.3	6.8	46.7	69.1	10.3	14.7	5.1	9.4

Figure 8 shows a graphical representation of the percentages per sleep phase during the first and the second half of the night.

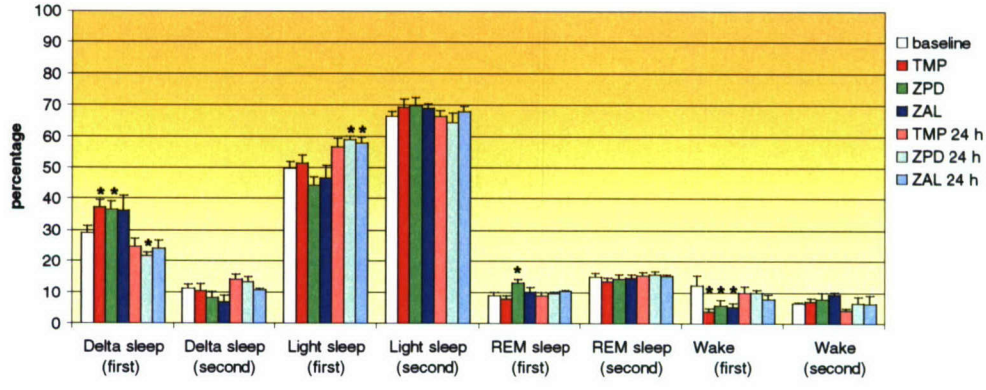

Figure 8 The percentage of observed sleep phases during the two parts of the night (first: before 0.00 h; second: after 0.00 h) + standard error of the mean. The data are plotted for the first (drug effect; dark colors) and the second night (recovery; light colors). Asterisks indicate statistical significances compared to baseline (see Table 5).

The observed percentages were statistically tested using independent T-tests. The significance levels are represented in Table 5.

Table 5 Results of the statistical analyses; black values indicating non-significances ($P > 0.05$), red values indicating significant effects ($P < 0.05$) and blue values indicating interesting trends.

	Delta first	Delta second	Light first	Light second	REM first	REM second	Wake first	Wake second
Baseline vs TMP	$P < 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P < 0.01$	$P > 0.05$
Baseline vs ZPD	$P < 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P < 0.01$	$P > 0.05$	$P < 0.05$	$P > 0.05$
Baseline vs ZAL	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P < 0.05$	$P > 0.05$
Baseline vs TMP 24 h	$P > 0.05$	$P > 0.05$	$P = 0.06$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
Baseline vs ZPD 24 h	$P < 0.01$	$P > 0.05$	$P < 0.001$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
Baseline vs ZAL 24 h	$P > 0.05$	$P > 0.05$	$P < 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

Also, the spindle activity in the recordings was observed. Spindles were increased after all drugs, but most remarkably after TMP. These data were not quantified due to the

labour intensity of such analyses, and therefore no further data on this matter is presented.
The results show that the sleep inducing drugs, with a short-lasting mechanism of action, have no effects on the distribution of the sleep phases in the second half of the night (after 0.00 h). However, the drugs do affect the sleep in a positive manner in the first half of the night as expected:

- the amount of Delta sleep increased after the sleep inducing drugs TMP and ZPD;
- also the amount of wakefulness decreased;
- there were only slight effects of ZPD on the amount of REM sleep;
- a large increase in spindle activity was observed after TMP (data not shown).

Also, some drugs affected the sleep during the first half of the following night:

- the amount of Delta sleep decreased after ZPD;
- the amount of light sleep increased after ZPD and ZAL;
- there were no effects on the amount of REM sleep or wakefulness.

The effects on the following night have also been observed in humans and are referred to as a so-called 'rebound'-effect in the literature. Also, after the drugs some so-called carry-over effects were observed the following morning, i.e. animals tended to fall asleep again. This effect was most pronounced after TMP.

5 Discussion

There are different possible solutions for sleep and alertness management in crew endurance plans [Simons and Valk, 1999]. One of these is the use of sleep-inducing and wake-promoting drugs. Hypnotic drugs are a class of drugs that induce sleep, used in the treatment of insomnia and in surgical anesthesia. Often the treatment of insomnia will not begin with drugs at all however, as many (not all) hypnotic drugs are habit forming. A physician will usually recommend alternatives before prescribing medication for sleep. This is due to a large number of factors known to disturb the human sleep pattern.

In situations when sleep needs to be induced by means of drugs, it is important to know that the induced sleep is not of a lower quality than normal sleep. The quality of sleep can be investigated by means of hypnograms by Rechtschaffen and Kales [1968] and the investigation of the sleep architecture. In the report of Busker *et al.* [2000] an overview of relevant literature concerning the effects of sleep inducers is given. Most of the classical hypnotics like barbiturates disturb the normal sleep pattern like the % of the REM sleep or the deep sleep stage.

In this study, the marmoset monkey model was validated using nocturnal electroencephalogram measurements. In order to test whether the proposed sleep inducing drugs affect the quality of sleep and/or disrupt the normal sleep cycle/architecture, the effects of the short acting hypnotic drugs temazepam, zolpidem and zaleplon on sleep parameters were determined in the marmoset monkey.

5.1 Validation of the model

Marmoset monkeys (*Callithrix jacchus*) sleep mono-phasically during the night and have very similar sleep EEG variables to human, including a sleep intensity decline during the night, regular REM sleep episodes with relatively long cycle duration.

The hypnograms and the calculated percentages of occurrence for the different sleep phases indeed show that the sleep architecture of the marmoset monkey is characterized by sleep cycles, as is the case for humans. However, the duration of these sleep cycles in marmoset monkey (approximately 45 minutes) is shorter, and occur more frequent than that of humans (approximately 90 minutes). These observations in the monkey model are in agreement with those of Crofts *et al.* [2001], who observed sleep cycle durations of about 50 minutes.

In general, the sleep of marmoset monkeys, despite existing of shorter sleep cycles, is similar to that of humans (see also Philippens *et al.* [2004]). Therefore, the marmoset monkey can be considered to be a valid model for the investigation of effects of sleep inducing drugs on the quality of sleep.

5.2 Effects of the sleep inducing drugs

All of the sleep inducing drugs were effective, the animals went to sleep quickly after administration even before the lights went off. The percentage of wakefulness during

the first half of the night (the time when the drug effects were expected to be maximal) was considerably decreased, i.e. the animals slept more after the sleep inducing drugs. However, all drugs showed some carry-over effects, i.e. animals tended to fall asleep again. These were most pronounced after TMP. This might indicate that especially TMP might affect the alertness and performance the following morning. In some cases after ZPD (2 out of 6 animals) and ZAL (1 out of 6 animals) such carry-over effects were also observed, but were not found to significant. Accordingly, in humans practically no carry-over effects were observed after ZAL [Zammit and Kramer 2001; O'Hanlon, 2002], however studies in humans show that TMP is unlikely to produce residual effects which conflicts with the observations in marmoset monkeys [review in Vermeeren, 2004]. Interestingly, after ZPD no carry over effects were observed in men, while in women ZPD caused significant residual sedation [Nicholson and Stone, 1999].

Another effect was the increase in the percentage of delta sleep during the first half of the night (before midnight), observed after TMP and ZPD (ZAL also seemed to have some effect, see Figure 7). Accordingly, similar effects were observed in rats after ZAL and in humans after ZPD [Kanno *et al.*, 2000; Noguchi *et al.*, 2004]. These effects can be considered to be positive effects since in general the quality of sleep is associated with the amount of delta sleep [Keklund and Åkerstedt, 1997]. It might be concluded that after the sleep inducing drugs the animals did not only sleep more, but also had a high(er) quality of sleep. Notably, after TMP a large amount of sleep spindles were observed. Sleep spindles are generally believed to indicate a state of unresponsiveness to sensory input [Steriade, 2000]. The facilitation of sleep spindles by TMP might then indicate that the chance of awakening due to sensory input is lowered, which is also a positive effect.

The night following the night of administration there was a decrease in the amount of delta sleep during the first half of the night, after ZPD. Also, the night following the night of administration, after ZPD and ZAL the occurrence of light sleep during the first half of the night was increased. These effects reflect so-called 'rebound-effects' or 'rebound insomnia' which is unwanted, but commonly observed after drug treatments. The most used definition of rebound insomnia is a significant worsening of sleep parameters after cessation of treatment as compared to baseline [review in Oude Voshaar *et al.*, 2004]. Similar to the effects observed in marmoset monkeys, rebound insomnia was observed in humans after ZPD [Oude Voshaar *et al.*, 2004]. In some cases rebound insomnia was observed after TMP and ZAL in humans, however mostly after high doses of the compounds [reviews in Dikeos and Soldatos, 2002; Wagner and Wagner, 2000].

For the choice in the ideal compound for the induction of sleep also the more practical issues come into play. Regarding ZAL there is the practical issue of availability, namely, despite the approval by the CBG (College ter Beoordeling van Geneesmiddelen) ZAL is not yet licensed for sale in The Netherlands.

6 Conclusion

In this study, the marmoset monkey model was validated using nocturnal electroencephalogram measurements for measuring effects on sleep. In order to test whether the proposed sleep inducing drugs affect the quality of sleep and/or disrupt the normal sleep cycle/architecture the effects of the short acting hypnotic drugs TMP, ZPD and ZAL on sleep were determined in the marmoset monkey.

The present study showed that the marmoset monkey model is a valid model for the research of sleep. Therefore, the effects on the quality of sleep after the sleep inducing drugs can be extrapolated towards the human situation.

In general, there were no large differences between the effects of the tested pre-selected sleep inducing drugs on the quality of sleep. All three drugs tended to affect the quality of sleep in a positive way, but also induced some 'carry-over effects'. TMP did show the most pronounced carry-over effects, which is unfavorable, but it also increased spindle activity which might be favorable. ZAL and ZPD induced some 'rebound-effects' on the sequent night. Also, literature suggests that ZPD leads to residual sleepiness in women. This might indicate that for the management of sleep in a military setting the sleep inducing drugs ZAL and TMP might be preferred over ZPD.

However, despite possessing the most favourable effects on the sleep pattern for sleep- and alertness management, it does not necessarily mean that TMP also have the most favourable effects on performance nor does this study address other possible side effects on other safety issues. Therefore, in a consecutive report (TNO-DV 2006 A270) the behavioral effects of the selected hypnotics are discussed [Philippens *et al.* 2006].

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8 Signature

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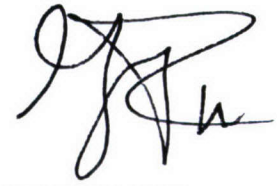

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15. ABSTRACT (MAXIMUM 200 WORDS (1044 BYTE))

The marmoset monkey model was validated using electroencephalogram -EEG- measurements for evaluating effects on sleep quality. In order to test whether the proposed hypnotics affect the quality of sleep and disrupt the normal sleep architecture, the effects of the short acting hypnotics temazepam, zolpidem and zaleplon on sleep were determined. The results showed that the marmoset monkey model is a valid model for the research of sleep. Furthermore, no large differences between the effects of the tested sleep inducing drugs on the quality of sleep could be observed. All drugs tended to affect the quality of sleep in a positive way. However, all drugs, temazepam especially, resulted in some carry-over effects, i.e. after awakening animals tended to fall asleep again. On the other hand, after temazepam sleep spindles were observed often. These spindles lower the chance of awakening as a result of sensory input like noise. On the other hand, zaleplon and zolpidem induced some 'rebound-effects'. Furthermore, there some indications that zolpidem has unwanted effects in women. This might indicate that for the management of sleep in a military setting the sleep inducing drugs temazepam and zaleplon might both be useful, with the preference for temazepam.

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